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PCT/NZ2005/000052

CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 22 March 2004 with an application for Letters Patent number 531866 made by Nicolai Vladimirovich Bovin; Lissa Gwyneth Gilliver; Stephen Michael Henry and Elena Yurievna.

I further certify that pursuant to a claim under Section 24(1) of the Patents Act 1953, a direction was given that the application proceed in the name of KIWI INGENUITY LIMITED.

Dated 3 May 2005.

Neville Harris
Commissioner of Patents, Trade Marks and Designs

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Patents Act 1953

PROVISIONAL SPECIFICATION

SYNTHETIC MEMBRANE ANCHORS

We, **Nicolai Vladimirovich BOVIN**, a Russian citizen of 117437 Moscow, Artsimovicha st. 11-181, Russian Federation; **Lissa Gwyneth GILLIVER**, a New Zealand citizen of 134c Church Street, Onehunga, Auckland, New Zealand; **Stephen Michael HENRY**, a New Zealand citizen of 18 Gracechurch Drive, Howick, Auckland, New Zealand; and **Elena YURIEVNA**, a Russian citizen of 117218, Novocheremushkinkaya str. 21-1-26, Moscow, Russian Federation; do hereby declare this invention to be described in the following statement:

SYNTHETIC MEMBRANE ANCHORS

FIELD OF INVENTION

The invention relates to water soluble synthetic molecules that spontaneously and stably incorporate into lipid bi-layers, including cell membranes. Particularly, although not exclusively, the invention relates to the use of these synthetic molecules as membrane anchors for antigens expressed at the cell surface.

BACKGROUND

Cell surface antigens mediate a range of interactions between cells and their environment. These interactions include cell-cell interactions, cell-surface interactions and cell-solute interactions. Cell surface antigens also mediate intra-cellular signalling.

Cells are characterised by qualitative and quantitative differences in the cell surface antigens expressed. Qualitative and quantitative changes in the cell surface antigens expressed alter both cell function (mode of action) and cell functionality (action served).

Being able to effect qualitative and/or quantitative changes in the surface antigens expressed by a cell has diagnostic and therapeutic value.

Cells exist in an aqueous environment. The cell membrane is a lipid bilayer that serves as a semi-permeable barrier between the cytoplasm of the cell and this aqueous environment.

Localising antigens to the cell surface may be achieved by the use of glycolipids as membrane anchors. The natural occurrence of cell surface antigens localised to the cell surface by means of glycolipid membrane anchors is well known.

Isolation of glycolipid-linked antigens and their incorporation into cell membranes to alter the characteristics of a cell has been reported. More recently the preparation of exogenously prepared glycolipid-linked antigens has been reported.

In all these reports the methods include the isolation of a glycolipid or glycolipid-linked antigen from a biological source. The isolation of glycolipids or glycolipid-linked antigens from biological sources is costly, variable and isolatable amounts are often limited.

Obtaining reagents from zoological sources for diagnostic or therapeutic use is problematic where the reagent or its derivative is to be administered to an individual or species of organism different from the source of the reagent. The problem is particularly acute when administration of the reagent or its derivative to a human subject is contemplated.

Synthetic molecules for which the risk of contamination with zoo-pathogenic agents can be excluded are therefore preferred. Synthetic counterparts for naturally occurring glycolipids and synthetic neo-glycolipids have been reported.

Glycolipids are able to spontaneously and stably incorporate into a lipid bi-layer from an aqueous environment. However, the utility of glycolipid-linked antigens for diagnostic or therapeutic purposes is limited to those glycolipid-linked antigens that will form a solution in saline.

Organic solvents and/or detergents used to facilitate the solubilization of glycolipid-linked antigens in saline must be biocompatible. Solvents and detergents must often be excluded or quickly removed as they can be damaging to some cell membranes. Damage to cell membranes is to be avoided especially where the supply of cells is limited, e.g. embryos.

Removal of solvents and detergents is also required if the preparation is to be administered to an individual as a diagnostic or therapeutic preparation. The removal of solvents or detergents from such preparations can be problematic.

There exists a need for water soluble synthetic molecules that are functionally equivalent to naturally occurring glycolipids and glycolipid-linked antigens in respect of their ability to spontaneously and stably incorporate into lipid bi-layers, including cell membranes.

Providing such synthetic molecules obviates the limitations of glycolipids and glycolipid-linked antigens isolated from zoological sources and facilitates being able to effect qualitative and/or quantitative changes in the surface antigens expressed by a cell by allowing for the use of organic solvents and/or detergents to be excluded.

It is an object of this invention to provide such synthetic molecules and a method for their preparation. It is a further object of this invention to provide diagnostic and therapeutic methods employing the use of such synthetic molecules. The preceding objects are to be read disjunctively with the object to at least provide the public with a useful choice.

STATEMENTS OF INVENTION

Accordingly in a **first** aspect the invention may broadly be said to consist in a water soluble synthetic molecule of the structure:

$$F\text{-}S_1\text{-}S_2\text{-}L$$

where:

F is an antigen selected from the group consisting of carbohydrates, proteins, lipids and chemically reactive functional groups;
S_1-S_2 is a spacer linking F to L; and
L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids.

Preferably the synthetic molecule spontaneously incorporates into a lipid bi-layer when a solution of the molecule is contacted with the lipid bi-layer.

Preferably F is selected from the group consisting of naturally occurring or synthetic glycotopes, antibodies (immunoglobulins), lectins, avidin, and biotin. More preferably F is a naturally occurring or synthetic glycotope consisting of three (trisaccharide) or more sugar units. Most preferably F is a naturally occurring glycotope selected from the group consisting of lacto-neo-tetraosyl, lactotetraosyl, lacto-nor-hexaosyl, lacto-iso-octaosyl, globoteraosyl, globo-neo-tetraosyl, globopentaosyl, gangliotetraosyl, gangliotriaosyl, gangliopentaosyl, isoglobotriaosyl, isoglobotetraosyl, mucotriaosyl and mucotetraosyl series of oligosaccharides.

When F is an oligosaccharide, L is a glycerophospholipid and S_2 is $CO(CH_2)_4CO$- (i.e. A is *bis*(N-hydroxysuccinimidyl) adipate), preferably S_1 is a C_{3-5}-aminoalkyl selected from the group consisting of: 3-aminopropyl, 4-aminobutyl, or 5-aminopentyl). More preferably S_1 is 3-aminopropyl.

In one embodiment F is selected from the group of glycotopes comprising the terminal sugars GalNAcα1-3(Fucα1-2)Galß; Galα1-3Galß; Galß; Galα1-3(Fucα1-2)Galß; NeuAcα2-3Galß; NeuAcα2-6Galß; Fucα1-2Galß; Galß1-4GlcNAcß1-6(Galß1-4GlcNAcß1-3)Galß; Fucα1-2Galß1-4GlcNAcß1-6(Fucα1-2Galß1-4GlcNAcß1-3)Galß; Fucα1-2Galß1-4GlcNAcß1-6(NeuAcα2-3Galß1-4GlcNAcß1-3)Galß; NeuAcα2-3Galß1-4GlcNAcß1-6(NeuAcα2-3Galß1-4GlcNAcß1-3)Galß; Galα1-4Galß1-4Glc; GalNAcß1-3Galα1-4Galß1-4Glc; GalNAcα1-3GalNAcß1-3Galα1-4Galß1-4Glc; or GalNAcß1-3GalNAcß1-3Galα1-4Galß1-4Glc.

In another embodiment F is a molecule that mediates a cell-cell or cell-surface interaction. Preferably F is carbohydrate, protein or lipid with an affinity for a component expressed on a targeted cell or surface. More preferably F has an affinity for a component expressed on epithelial cells or extra-cellular matrices. Most preferably F has an affinity for a component expressed on the epithelial cells or the extra-cellular matrix of the endometrium.

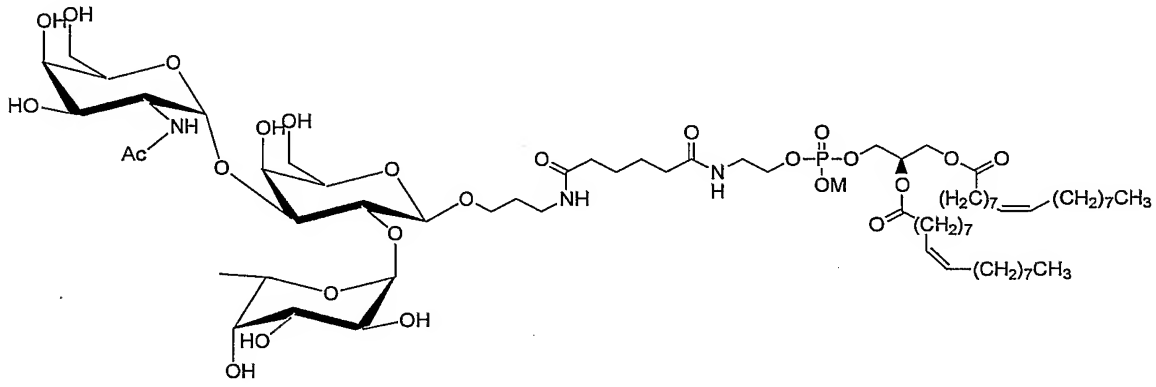
The component expressed on the epithelial cells or the extra-cellular matrix of the endometrium can be a naturally expressed component or an exogenously incorporated component.

In yet another embodiment F is a molecule that mediates a cell-solute interaction. Preferably F is a receptor for a ligand where the presence of the ligand is diagnostic for a pathological condition. More preferably F is an antibody (immunoglobulin) for the ligand (diagnostic molecule), or an antigen for the ligand where the ligand (diagnostic molecule) is an antibody.

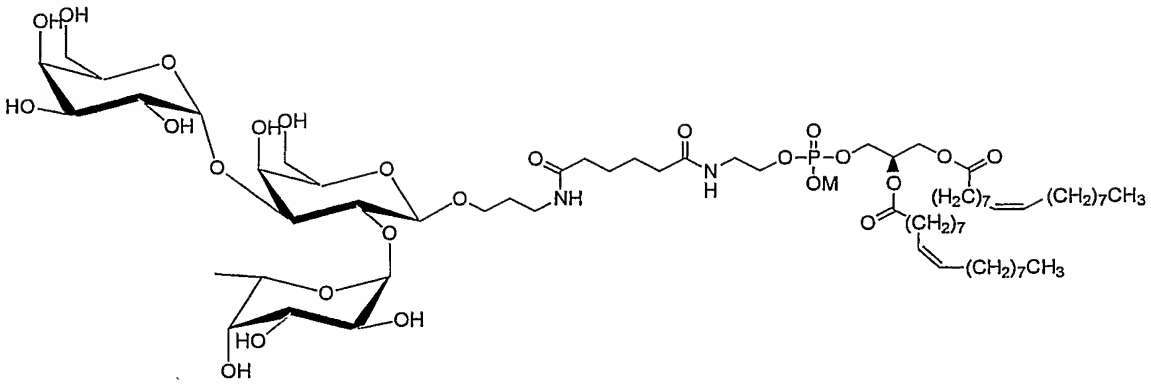
S_1-S_2 is selected to provide a water soluble synthetic molecule. When F is an oligosaccharide and L is a glycerophospholipid preferably S_1 is selected from the group including: primary aminoalkyl, secondary aliphatic aminoalkyl or primary aromatic amine, and S_2 is absent or selected from the group including: $-CO(CH_2)_3CO-$, $-CO(CH_2)_4CO-$ (adipate), $-CO(CH_2)_5CO-$. Most preferably S_1 is 3-aminopropyl and S_2 is $-CO(CH_2)_4CO-$ (adipate).

Preferably L is selected from the group consisting of: diacylglycerolipids, phosphatidate, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, phosphatidyl glycerol, and diphosphatidyl glycerol derived from one or more of *trans*-3-hexadecenoic acid, *cis*-5-hexadecenoic acid, *cis*-7-hexadecenoic acid, *cis*-9-hexadecenoic acid, *cis*-6-octadecenoic acid, *cis*-9-octadecenoic acid, *trans*-9-octadecenoic acid, *trans*-11-octadecenoic acid, *cis*-11-octadecenoic acid, *cis*-11-eicosenoic acid or *cis*-13-docsenoic acid. More preferably L is selected from the group consisting of: 1,2-O-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) and DSPE.

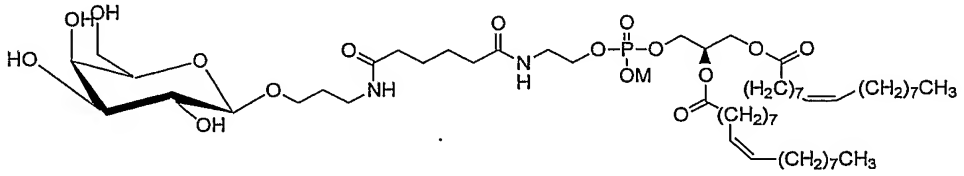
In specific embodiments the water soluble synthetic molecule has the structure:



designated A_{tri}-sp-Ad-DOPE (**I**); the structure designated A_{tri}-sp_1sp_2-Ad-DOPE (**II**); the structure designated A_{tri}-sp-Ad-DSPE (**III**);



designated B_{tri}-sp-Ad-DOPE (**VI**); the structure designated H_{tri}-sp-Ad-DOPE (**VII**); the structure designated H_{di}-sp-Ad-DOPE (**VIII**); or



designated Galß$_{l}$-sp-Ad-DOPE (**IX**).

In a **second** aspect the invention may broadly be said to consist in a method of preparing a water soluble synthetic molecule including the steps:

1. Reacting an activator (A) with a lipid (L) to provide an activated lipid (A-L);
2. Derivatising an antigen (F) to provide a derivatised antigen (F-S_1); and
3. Condensing A-L with F-S_1;

to provide the water soluble synthetic molecule of the structure:

F-S_1-S_2-L

where:

A is an activator selected from the group including: N-hydroxysuccinimide, *bis*(N-hydroxysuccinimidyl) adipate and 4-nitrophenol;
L is a lipid selected from the group consisting of diacyl- and dialkyl-glycerolipids, including glycerophospholipids;
F is an antigen selected from the group consisting of carbohydrates, proteins, lipids, and chemically reactive functional groups;
S_1-S_2 is a spacer linking F to L where:

S_1 is selected from the group including: primary aminoalkyl, secondary aliphatic aminoalkyl or primary aromatic amine; and
S_2 is absent or selected from the group including: $-CO(CH_2)_3CO-$, $-CO(CH_2)_4CO-$ (adipate), $-CO(CH_2)_5CO-$.

Preferably F is selected from the group consisting of naturally occurring or synthetic glycotopes, antibodies (immunoglobulins), lectins, avidin, and biotin. More preferably F is a naturally occurring or synthetic glycotope consisting of three or more sugar units (oligosaccharide). Most preferably F is a naturally occurring glycotope selected from the group consisting of lacto-neo-tetraosyl, lactotetraosyl, lacto-nor-hexaosyl, lacto-iso-octaosyl, globoteraosyl, globo-neo-tetraosyl, globopentaosyl, gangliotetraosyl, gangliotriaosyl, gangliopentaosyl, isoglobotriaosyl, isoglobotetraosyl, mucotriaosyl and mucotetraosyl series of oligosaccharides.

In one embodiment F is selected from the group of glycotopes comprising the terminal sugars GalNAcα1-3(Fucα1-2)Galß; Galα1-3Galß; Galß; Galα1-3(Fucα1-2)Galß; NeuAcα2-3Galß; NeuAcα2-6Galß; Fucα1-2Galß; Galß1-4GlcNAcß1-6(Galß1-4GlcNAcß1-3)Galß; Fucα1-2Galß1-4GlcNAcß1-6(Fucα1-2Galß1-4GlcNAcß1-3)Galß; Fucα1-2Galß1-4GlcNAcß1-6(NeuAcα2-3Galß1-4GlcNAcß1-3)Galß; NeuAcα2-3Galß1-4GlcNAcß1-6(NeuAcα2-3Galß1-4GlcNAcß1-3)Galß; Galα1-4Galß1-4Glc; GalNAcß1-3Galα1-4Galß1-4Glc; GalNAcα1-3GalNAcß1-3Galα1-4Galß1-4Glc; or GalNAcß1-3GalNAcß1-3Galα1-4Galß1-4Glc.

A and S_1 are selected to provide a water soluble synthetic molecule. When F is an oligosaccharide, L is a glycerophospholipid and S_2 is $CO(CH_2)_4CO-$ (i.e. A is *bis*(N-hydroxysuccinimidyl) adipate), preferably S_1 is a C_{3-5}-aminoalkyl selected from the group consisting of: 3-aminopropyl, 4-aminobutyl, or 5-aminobutyl). More preferably S_1 is 3-aminopropyl.

In another embodiment F is a molecule that mediates a cell-cell or cell-surface interaction. Preferably F is carbohydrate, protein or lipid with an affinity for a component expressed on a targeted cell or surface. More preferably F has an affinity for a component expressed on epithelial cells or extra-cellular matrix. Most preferably F has an affinity for a component expressed on the epithelial cells or the extra-cellular matrix of the endometrium.

In yet another embodiment F is a molecule that mediates a cell-solute interaction. Preferably F is a receptor for a ligand where the presence of the ligand is diagnostic for a pathological condition. More preferably F is an antibody for the ligand (diagnostic molecule), or an antigen for the ligand where the ligand (diagnostic molecule) is an antibody.

Preferably L is selected from the group consisting of: diacylglycerolipids, phosphatidate, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol,

phosphatidyl glycerol, and diphosphatidyl glycerol derived from one or more of *trans*-3-hexadecenoic acid, *cis*-5-hexadecenoic acid, *cis*-7-hexadecenoic acid, *cis*-9-hexadecenoic acid, *cis*-6-octadecenoic acid, *cis*-9-octadecenoic acid, *trans*-9-octadecenoic acid, *trans*-11-octadecenoic acid, *cis*-11-octadecenoic acid, *cis*-11-eicosenoic acid or *cis*-13-docsenoic acid. More preferably L is selected from the group consisting of: 1,2-O-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) and DSPE.

In a **third** aspect the invention may broadly be said to consist in a water soluble synthetic molecule prepared by a method according to the second aspect of the invention.

In a **fourth** aspect the invention may broadly be said to consist in a method of effecting qualitative and/or quantitative changes in the surface antigens expressed by a cell or multi-cellular structure including the step:

1. Contacting a suspension of the cell or multi-cellular structure with a solution of a water soluble synthetic molecule according to the first aspect or third aspect of the invention for a time and at a temperature sufficient to effect the qualitative and/or quantitative change in the surface antigens expressed by the cell or multi-cellular structure.

Preferably the cell or multi-cellular structure is of human origin.

In one embodiment the cell is a red blood cell.

In this embodiment preferably F is selected from the group of glycotopes comprising the terminal sugars GalNAcα1-3(Fucα1-2)Galß; Galα1-3Galß; Galß; Galα1-3(Fucα1-2)Galß; NeuAcα2-3Galß; NeuAcα2-6Galß; Fucα1-2Galß; Galß1-4GlcNAcß1-6(Galß1-4GlcNAcß1-3)Galß; Fucα1-2Galß1-4GlcNAcß1-6(Fucα1-2Galß1-4GlcNAcß1-3)Galß; Fucα1-2Galß1-4GlcNAcß1-6(NeuAcα2-3Galß1-4GlcNAcß1-3)Galß; NeuAcα2-3Galß1-4GlcNAcß1-6(NeuAcα2-3Galß1-4GlcNAcß1-3)Galß; Galα1-4Galß1-4Glc; GalNAcß1-3Galα1-4Galß1-4Glc; GalNAcα1-3GalNAcß1-3Galα1-4Galß1-4Glc; or GalNAcß1-3GalNAcß1-3Galα1-4Galß1-4Glc. More preferably F is selected from the group of glycotopes consisting of the oligosaccharides GalNAcα1-3(Fucα1-2)Galß and Galα1-3(Fucα1-2)Galß.

In this embodiment preferably the solution of the water soluble synthetic molecule has a concentration of at least 0.05 mg/mL.

In this embodiment preferably the solution of the water soluble synthetic molecule is contacted with the red blood cell for at least 1 hour at around 37 °C.

In another embodiment the multi-cellular structure is an embryo.

In this embodiment preferably F is an attachment molecule where the attachment molecule has an affinity for a component expressed on the epithelial cells or the extra-cellular matrix of the endometrium.

The component expressed on the epithelial cells or the extra-cellular matrix of the endometrium can be a naturally expressed component or an exogenously incorporated component.

In yet another embodiment the cell is red blood cell.

In this embodiment preferably F is a receptor for a ligand where the presence of the ligand is diagnostic for a pathological condition. More preferably F is an antibody (immunoglobulin) for the ligand (diagnostic molecule), or an antigen for the ligand where the ligand (diagnostic molecule) is an antibody.

In a **fifth** aspect the invention may broadly be said to consist in a cell or multi-cellular structure incorporating a water soluble synthetic molecule according to the first aspect or third aspect of the invention. Preferably the cell is a red blood cell incorporating a water soluble synthetic molecule selected from the group consisting of: A_{tri}-sp-Ad-DOPE (**I**); and B_{tri}-sp-Ad-DOPE (**VI**). More preferably the cell or multi-cellular structure is of human origin.

In a **sixth** aspect the invention may broadly be said to consist in a kit comprising a dried preparation or solution of a water soluble synthetic molecule according to the first aspect or third aspect of the invention. Preferably the water soluble synthetic molecule is selected from the group consisting of: A_{tri}-sp-Ad-DOPE (**I**); and B_{tri}-sp-Ad-DOPE (**VI**).

In a **seventh** aspect the invention may broadly be said to consist in a kit comprising a suspension of cells or multi-cellular structures according to the fifth aspect of the invention. Preferably the cells are red blood cells that do not naturally express A- or B-antigen and incorporate a water soluble synthetic molecule selected from the group consisting of: A_{tri}-sp-Ad-DOPE (**I**); and B_{tri}-sp-Ad-DOPE (**VI**). More preferably the cell or multi-cellular structure is of human origin.

In an **eighth** aspect the invention may broadly be said to consist in a pharmaceutical preparation comprising a dried preparation or solution of a water soluble synthetic molecule according to the first aspect or third aspect of the invention.

In one embodiment the pharmaceutical preparation is in a form for administration by inhalation.

In another embodiment the pharmaceutical preparation is in a form for administration by injection.

In a **ninth** aspect the invention may broadly be said to consist in a pharmaceutical preparation comprising a suspension of cells or multi-cellular structures according to the fifth aspect of the invention. Preferably the cell or multi-cellular structure is of human origin.

In one embodiment the pharmaceutical preparation is in a form for administration by inhalation.

In another embodiment the pharmaceutical preparation is in a form for administration by injection.

DETAILED DESCRIPTION

The synthetic molecules of the invention spontaneously incorporate into a lipid bi-layer, such as a membrane, when a solution of the molecule is contacted with the lipid bi-layer. Whilst not wishing to be bound by theory it is believed that the insertion into the membrane of the lipid tails of the lipid (L) is thermodynamically favoured. Subsequent disassociation of the synthetic molecule from the lipid membrane is believed to be thermodynamically unfavoured.

Accordingly the synthetic molecules of the invention are used to transform cells resulting in qualitative and/or quantitative changes in the surface antigens expressed. It will be recognised that the transformation of cells in accordance with the invention is distinguished from transformation of cells by genetic engineering. The invention provides for phenotypic transformation of cells *without* genetic transformation.

In the context of this description the term "transformation" in reference to cells is used to refer to the insertion or incorporation into the cell membrane of exogenously prepared synthetic molecules thereby effecting qualitative and quantitative changes in the cell surface antigens expressed by the cell.

The synthetic molecules of the invention comprise an antigen (F) linked to a lipid portion (or moiety) (L) via a spacer (S_1-S_2). The synthetic molecules can be prepared by the condensation of a primary aminoalkyl, secondary aliphatic aminoalkyl or primary aromatic amine derivative of the antigen with an activated lipid.

A desired phenotypic transformation may be achieved using the synthetic molecules of the invention in a one step method or a two step method. In the one step method the water soluble synthetic molecule (F-S_1-S_2-L) comprises the surface antigen as F.

In the two step method the water soluble synthetic molecule (F-S_1-S_2-L) comprises an antigen (F) that serves as a functional group to which the surface antigen can be linked following insertion of the synthetic molecule into the membrane. The functional group can be a group

such as avidin, biotin, a chelator or a chemically reactive functional group.

In accordance with the invention the primary aminoalkyl, secondary aliphatic aminoalkyl or primary aromatic amine and the activator of the lipid are selected to provide a synthetic molecule that is water soluble and will spontaneously incorporate into a lipid bi-layer when a solution of the synthetic molecule is contacted with the lipid bi-layer.

In the context of this description the phrase "water soluble" means a stable, single phase system is formed when the synthetic molecule is contacted with water or saline (such as PBS) in the absence of organic solvents or detergents, and the term "solution" has a corresponding meaning.

The synthetic molecules stably incorporate into the lipid bi-layer or membrane.

In the context of this description the phrase "stably incorporate" means that the synthetic molecules incorporate into the lipid bi-layer or membrane with minimal subsequent exchange between the lipid bi-layer or membrane and the external aqueous environment of the lipid bi-layer or membrane.

The selection of the primary aminoalkyl, secondary aliphatic aminoalkyl or primary aromatic amine and the activator depends on the physico-chemical properties of the antigen (F) to be linked to the lipid (L).

It will be understood by those skilled in the art that for a non-specific interaction, such as the interaction between a diacyl- or dialkyl-glycerolipid and a membrane, structural and stereo-isomers of naturally occurring lipids can be functionally equivalent. For example, it is contemplated by the inventors that diacylglycerol 2-phosphate could be substituted for phosphatidate (diacylglycerol 3-phosphate). Furthermore it is contemplated by the inventors that the absolute configuration of phosphatidate can be either R or S.

The inventors have determined that to prepare synthetic molecules of the invention where the antigen (F) is an oligosaccharide selected from the group of glycotopes for A-, B- and H-antigens of the ABO blood groups, the primary aminoalkyl, secondary aliphatic aminoalkyl or primary aromatic amine, and the activator should be selected to provide a spacer (S_1-S_2) with a structure according to one of those presented in Table 1.

It will be understood by one skilled in the art that once the structure of the spacer (S_1-S_2) has been determined for a given class of antigens, the same structure of the spacer can be adopted to prepared water soluble synthetic molecules of other classes of antigen with similar physico-chemical properties.

For example, the structure of the spacer for water soluble synthetic molecules (F-S_1-S_2-L) where F is a glycotope of the A-, B- and H-antigens of the ABO blood groups, may be the structure of the spacer selected to prepare water soluble synthetic molecules of other antigens with physico-chemical properties similar to the glycotopes of the A-, B- and H-antigens of the ABO blood groups.

Not all structures of the spacer (S_1-S_2) will provide a synthetic molecule (F-S_1-S_2-L) that is water soluble. In addition to the water soluble synthetic molecules of the invention the inventors have prepared synthetic molecules A_{tri}-sp-lipid (**IV**) and A_{tri}-PAA-PEA (**V**) that were determined not to be water soluble (Table 2).

In principle the glycotope of a broad range of blood group related glycolipids or glycoproteins could be made the antigen (F) of the water soluble synthetic molecule F-S_1-S_2-L where S_1-S_2-L is identical or equivalent to the corresponding portion of the water soluble synthetic molecules designated A_{tri}-sp-Ad-DOPE (**I**), A_{tri}-sp_1sp_2-Ad-DOPE (**II**), A_{tri}-sp-Ad-DSPE (**III**), B_{tri}-sp-Ad-DOPE (**VI**), H_{tri}-sp-Ad-DOPE (**VII**), H_{di}-sp-Ad-DOPE (**VIII**), and Galß-sp-Ad-DOPE (**IX**).

It will be understood by those skilled in the art that the water soluble synthetic molecules (F-S_1-S_2-L) of the invention where F is an oligosaccharide may be used as "synthetic glycolipids" and substituted for glycolipids obtained from biological (botanical or zoological) sources.

The structures of known blood group-related glycolipids and glycoproteins (see references) are provided in the following list:

<u>Glycolipids*</u>

(*In general, for almost all examples of A-antigens the terminal A sugar GalNAc can be replaced with the B sugar Gal. Additionally, the lack of either the A or B determinant creates the equivalent H determinant.)

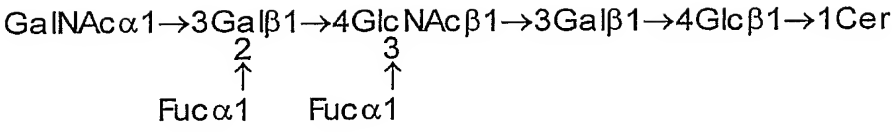
A-6-1

$$\text{GalNAc}\alpha1\rightarrow3\text{Gal}\beta1\rightarrow3\text{GlcNAc}\beta1\rightarrow3\text{Gal}\beta1\rightarrow4\text{Glc}\beta1\rightarrow1\text{Cer}$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 2\uparrow$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \text{Fuc}\alpha1$$

A-6-2

$$\text{GalNAc}\alpha1\rightarrow3\text{Gal}\beta1\rightarrow4\text{GlcNAc}\beta1\rightarrow3\text{Gal}\beta1\rightarrow4\text{Glc}\beta1\rightarrow1\text{Cer}$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 2\uparrow$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \text{Fuc}\alpha1$$

A-7-2 (ALe^y)



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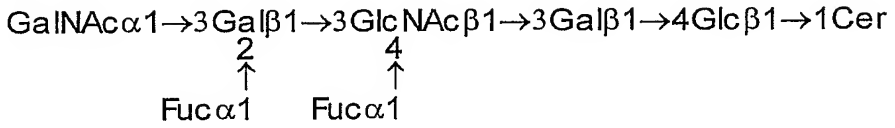
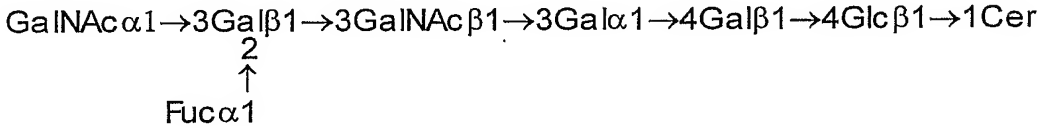
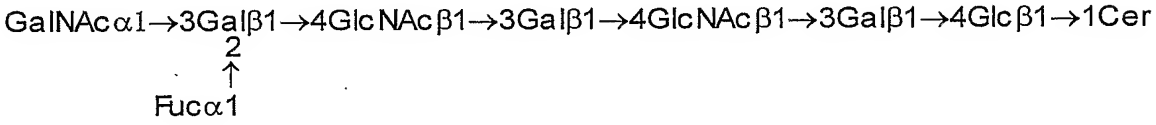


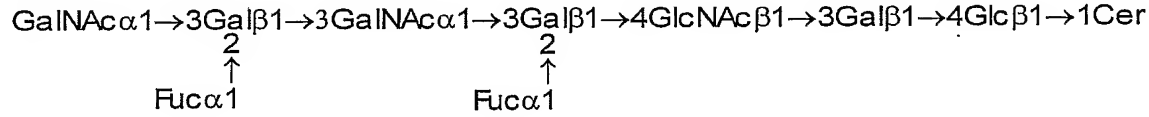
A-7-4



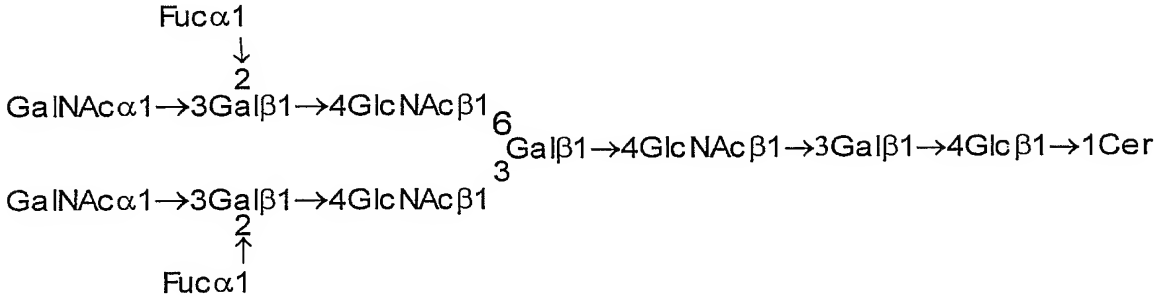
A-8-2



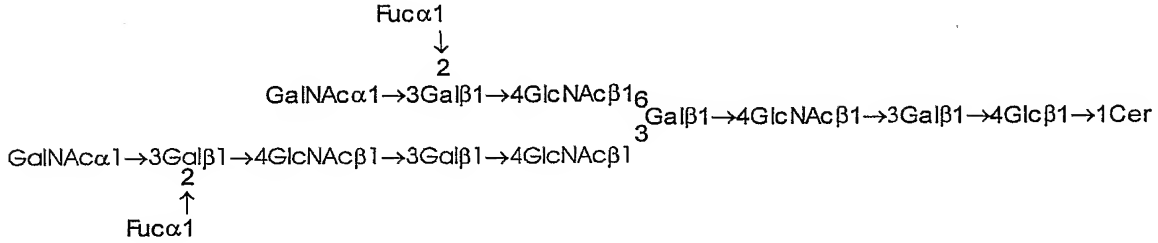
A-9-3



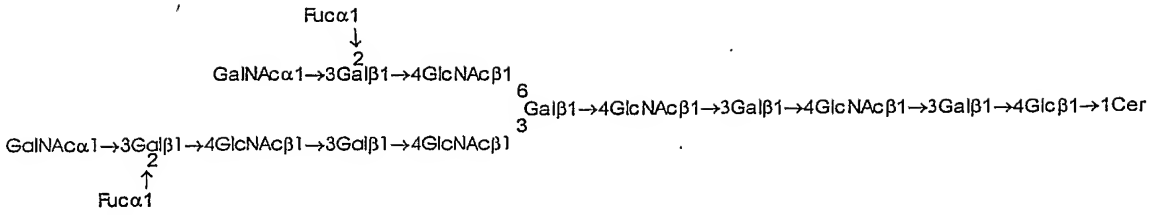
A-12-2



A-14-2



A-16-2



Lactosylceramide

$$Gal\beta1\rightarrow4Glc\beta1\rightarrow1Cer$$

Hematoside/G_{M3}

$$NeuAc\alpha2\rightarrow3Gal\beta1\rightarrow4Glc\beta1\rightarrow1Cer$$

Lactotriaosylceramide

$$GlcNAc\beta1\rightarrow3Gal\beta1\rightarrow4Glc\beta1\rightarrow1Cer$$

Globotriaosylceramide/P^K

$$Gal\alpha1\rightarrow4Gal\beta1\rightarrow4Glc\beta1\rightarrow1Cer$$

Globoside/P

$$GalNAc\beta1\rightarrow3Gal\alpha1\rightarrow4Gal\beta1\rightarrow4Glc\beta1\rightarrow1Cer$$

Paragloboside/neolactotetraosylceramide

$$Gal\beta1\rightarrow4GlcNAc\beta1\rightarrow3Gal\beta1\rightarrow4Glc\beta1\rightarrow1Cer$$

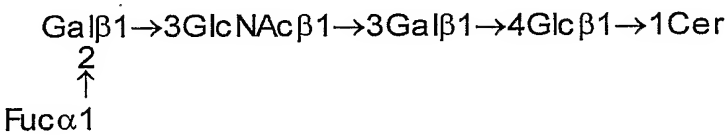
Le^c-4/Lactotetraosylceramide

$$Gal\beta1\rightarrow3GlcNAc\beta1\rightarrow3Gal\beta1\rightarrow4Glc\beta1\rightarrow1Cer$$

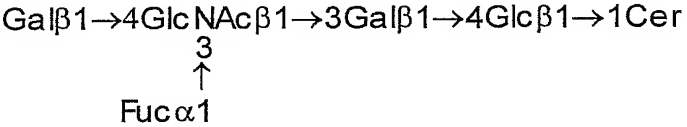
Sialoyl paragloboside/sialoyl neolactotetraosylceramide

$$NeuAc\alpha2\rightarrow3Gal\beta1\rightarrow4GlcNAc\beta1\rightarrow3Gal\beta1\rightarrow4Glc\beta1\rightarrow1Cer$$

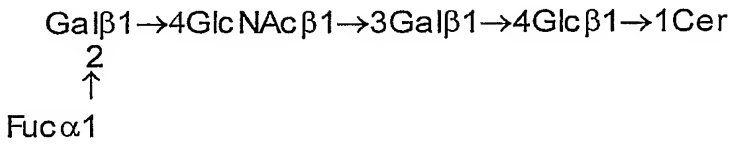
H-5-1



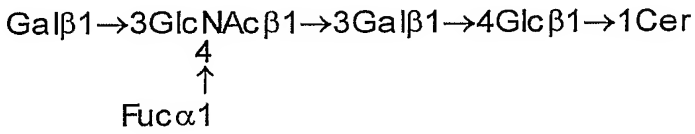
Le^x-5



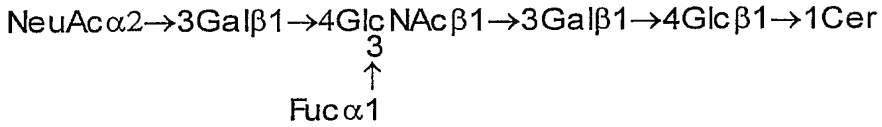
H-5-2



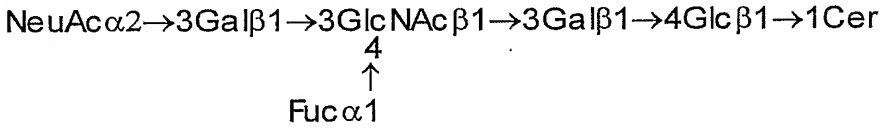
Le^a-5



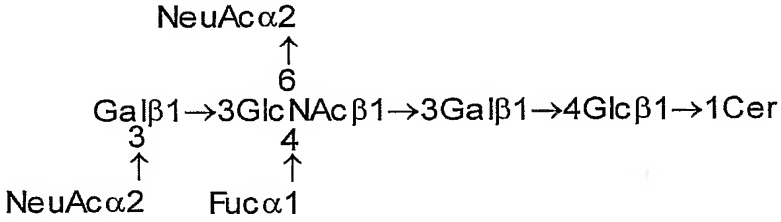
Sialoyl Le^x



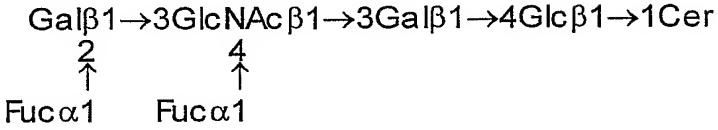
Sialoyl Le^a-6/gastrointestinal cancer antigen (GICA or Ca 19-9)



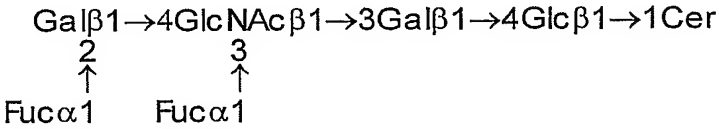
Disialoyl Le^a-7



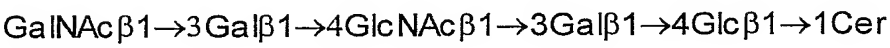
Le^b-6



Le^y-6



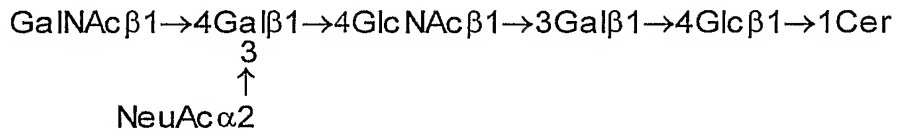
P-like



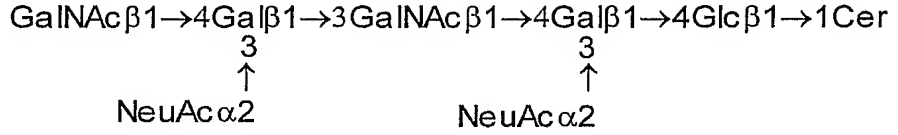
Forssman antigen

GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer

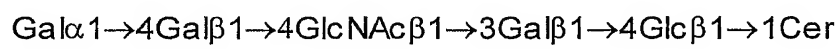
Cad erythrocyte



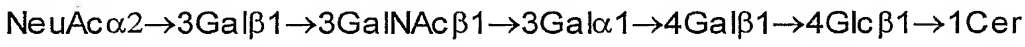
Cad hepato-carcinoma antigen



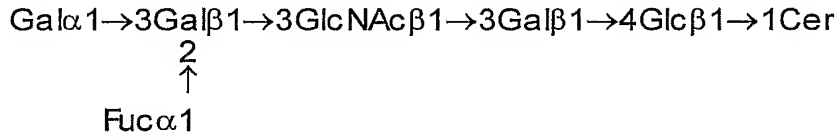
P_1



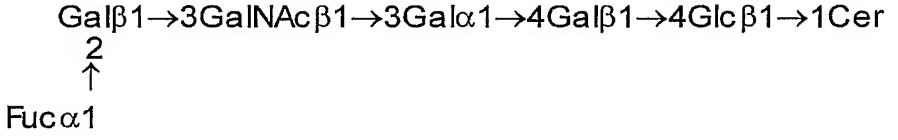
LKE/'GL 7/SSEA-4



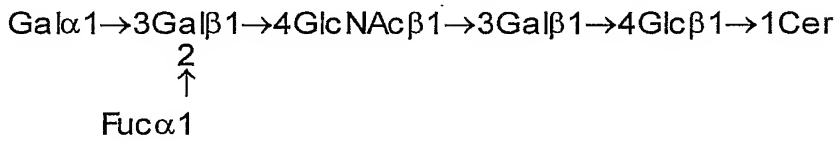
B-6-1



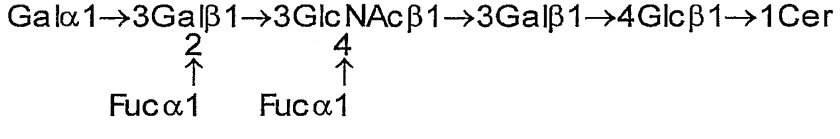
H-6-4



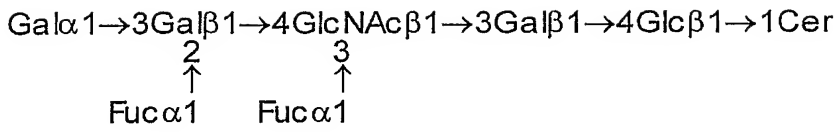
B-6-2



BLe^b-7



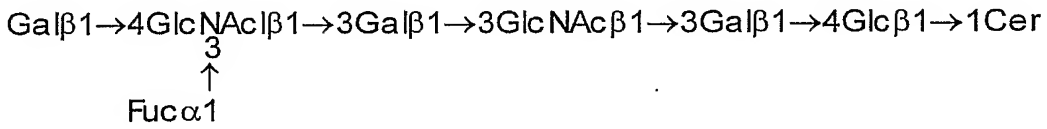
BLe^y-7



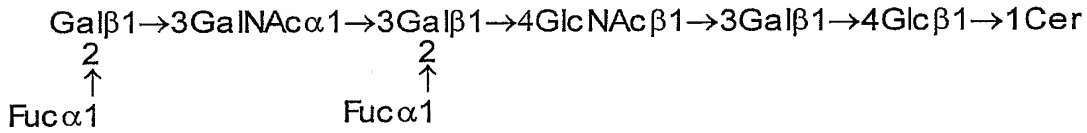
i antigen/lacto-N-*nor*-hexaosylceramide

Sialoyl-*nor*-hexaosylceramide/sialoyl-lacto-N-*nor*-hexaosylceramide

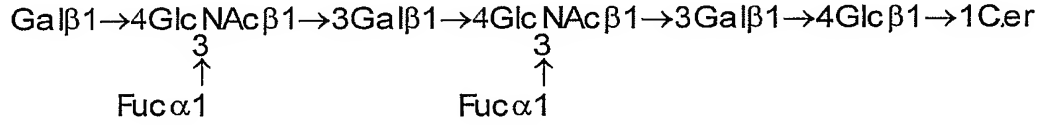
Le^x-7



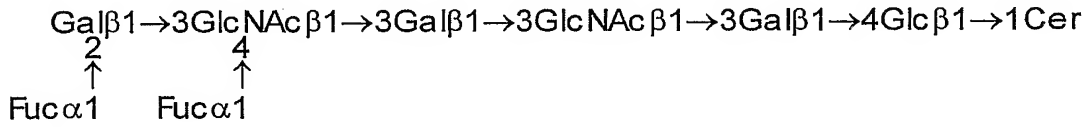
H-8-3



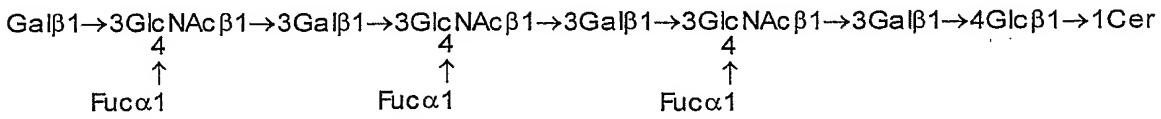
Le^x-8



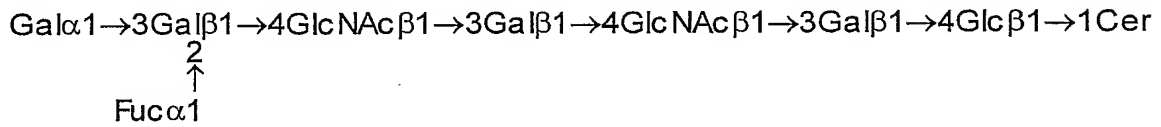
Le^b-8



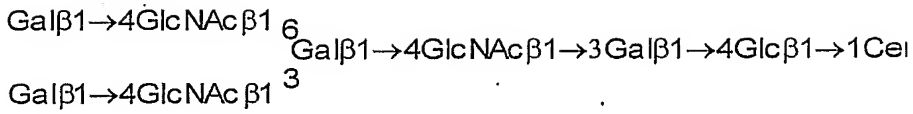
Le^a-11



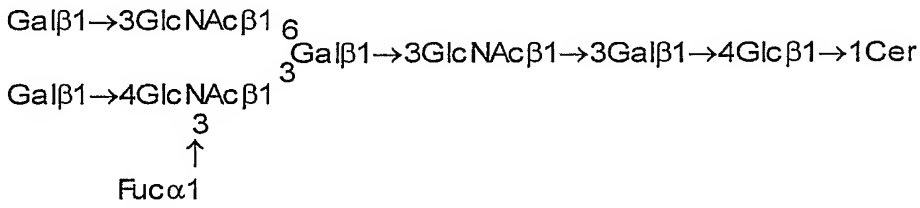
B-8-2



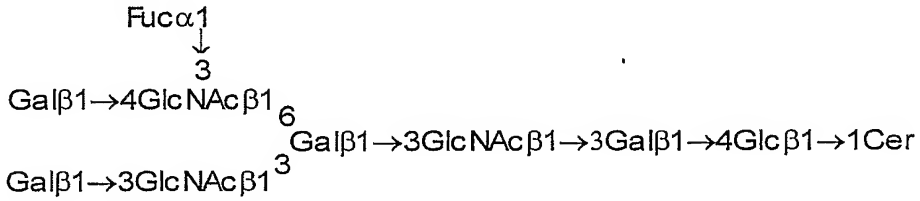
I antigen



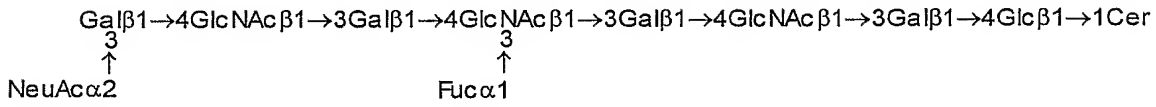
Le^c-9 (fucosylated backbone)



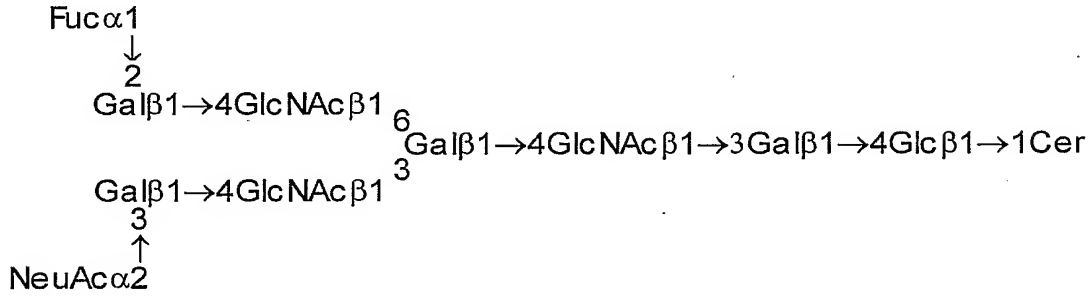
Le^c-9 (fucosylated branch)



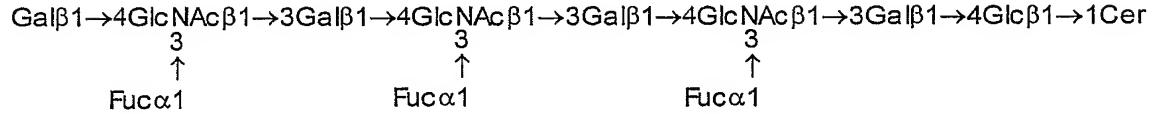
VIM-2



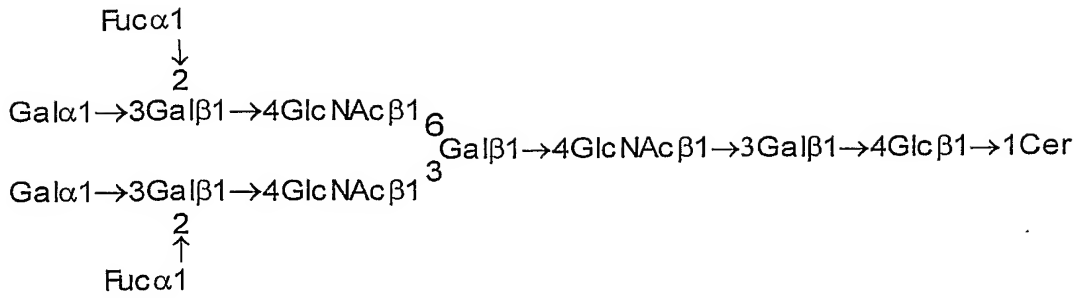
Erythrocyte FI antigen



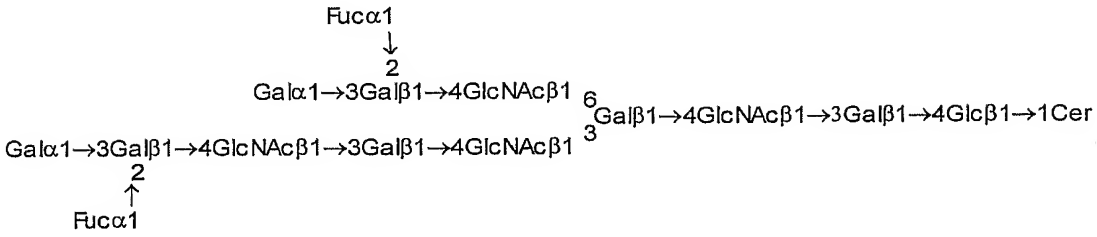
Le^x-11



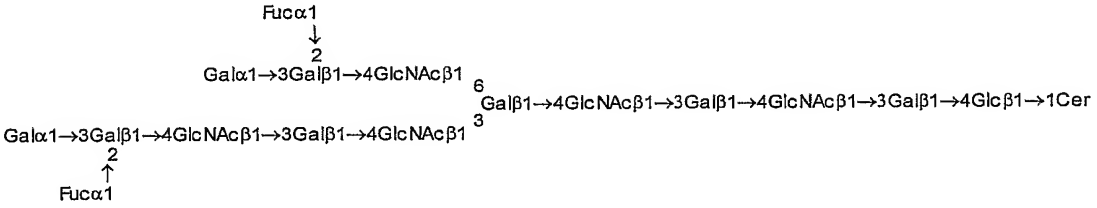
B-12-2



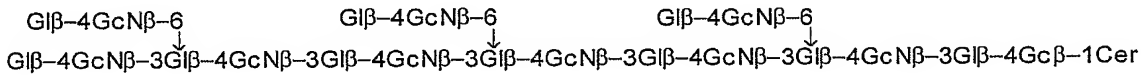
B-14-2



B-16-2

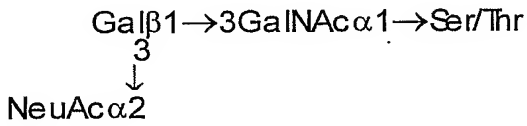


I-active polyglycosylceramide

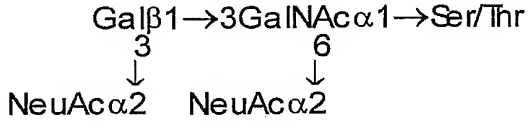


<u>O-linked Glycoproteins</u>

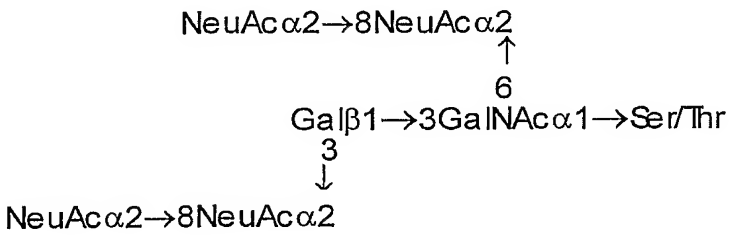
Monosialotrisaccharide



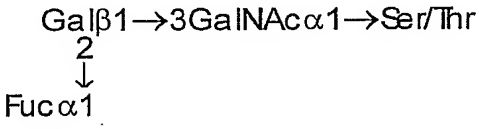
Disialotetrasaccharide



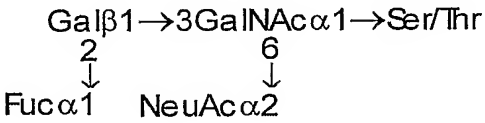
Disialoyl group oligosaccharide



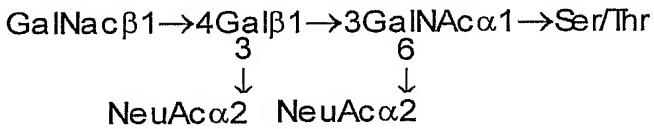
H-active trisaccharide



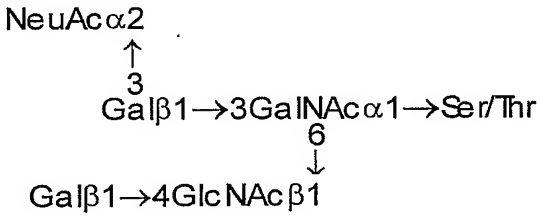
Sialylated H-active tetrasaccharide



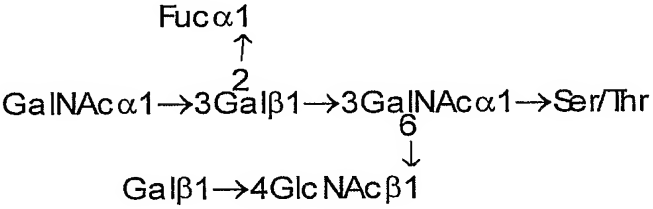
Cad oligosaccharide



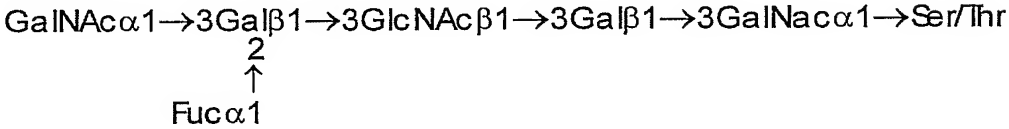
GlcNAc oligosaccharide



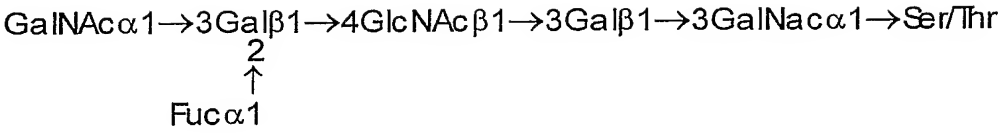
Mucin oligosaccharide/A-active glycoprotein



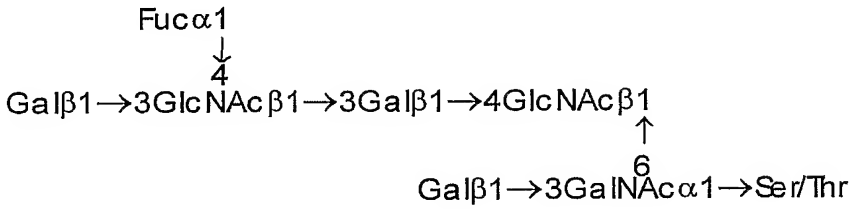
Ovarian cyst A-active glycoprotein-6a



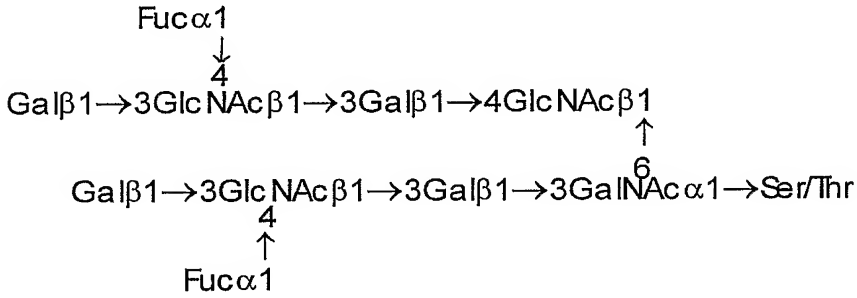
Ovarian cyst A-active glycoprotein-6b



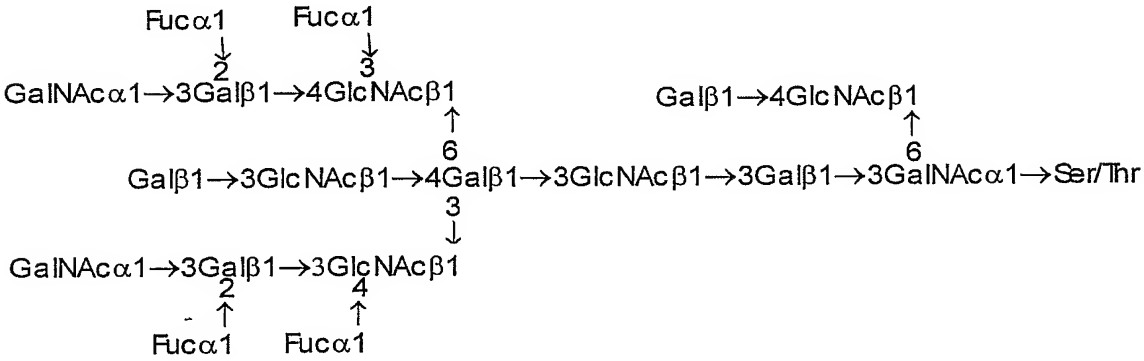
Ovarian cyst Le^a-active glycoprotein-7



Ovarian cyst Le^a-active glycoprotein-10

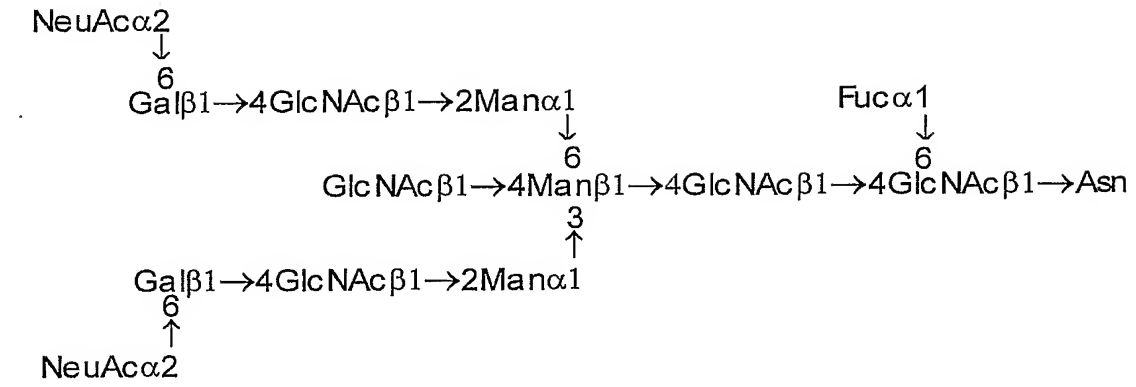


Ovarian cyst A-active glycoprotein-18

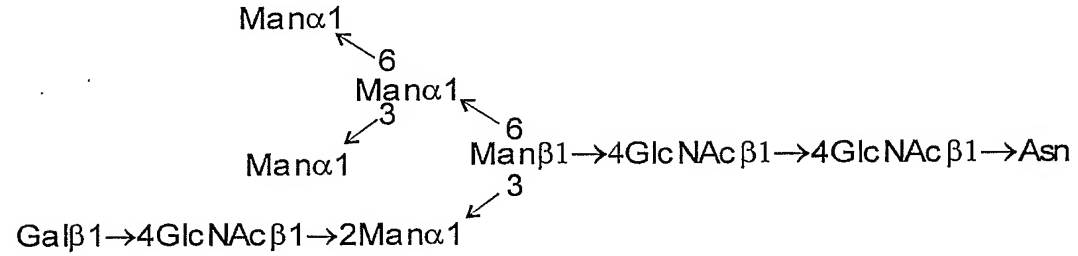


<u>N-linked Glycoproteins</u>

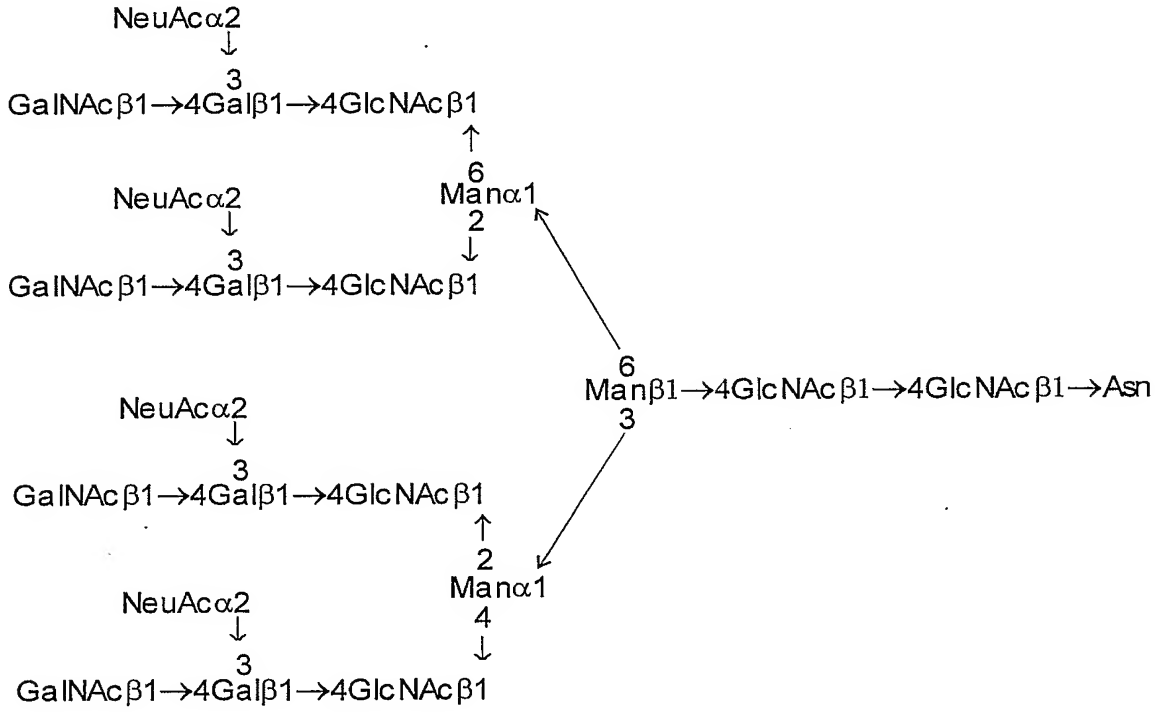
Complex type/Alkali-stable chain



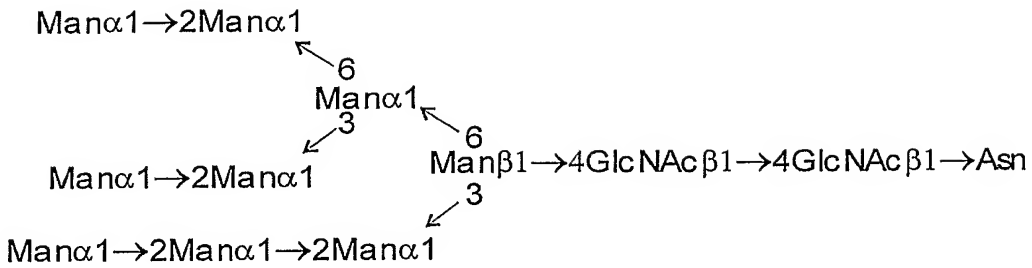
Hybrid type



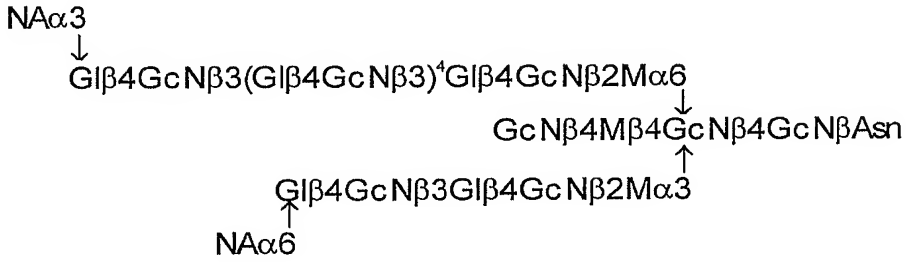
Tamm-Horsfall glycoprotein



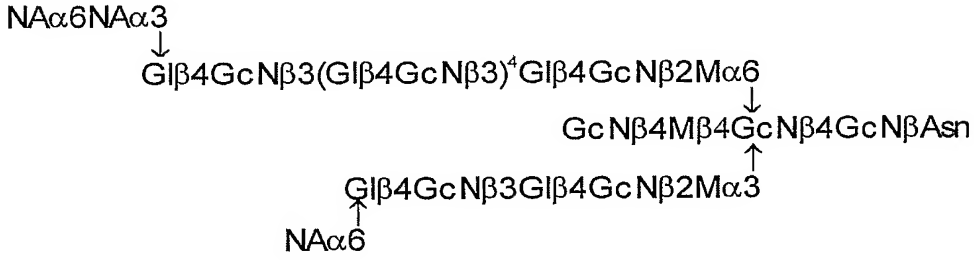
High-mannose type



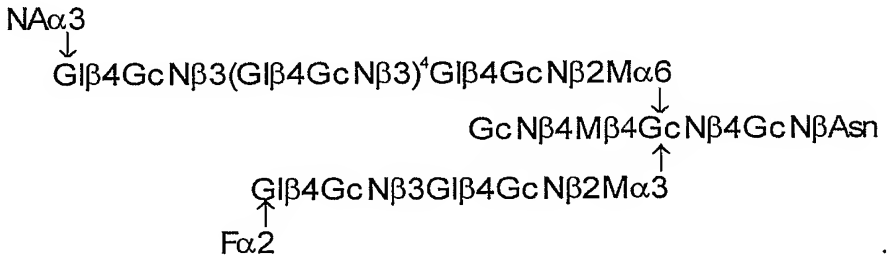
Disialoyl foetal erythrocyte antigen



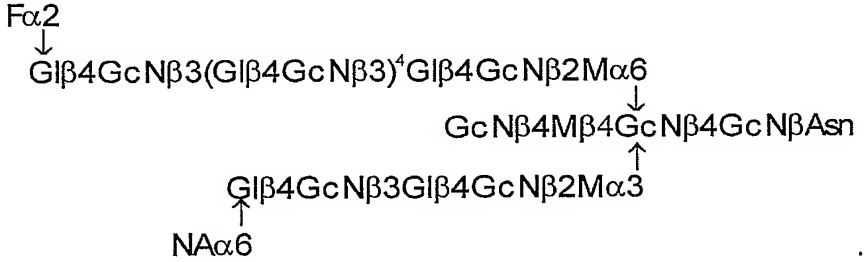
Trisialoyl foetal erythrocyte antigen (disialoyl group on branch)



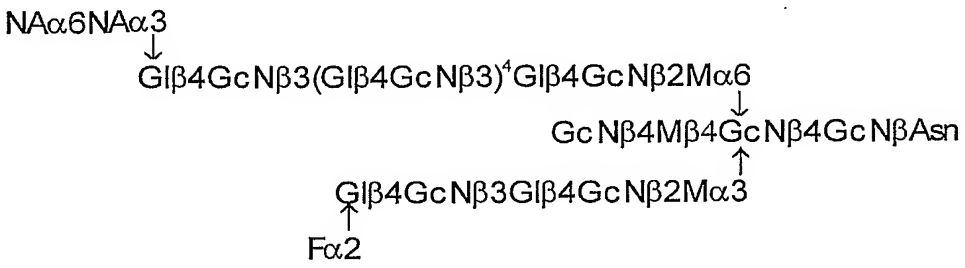
Monofucosyl-monosialoyl foetal erythrocyte antigen (fucosylated backbone)



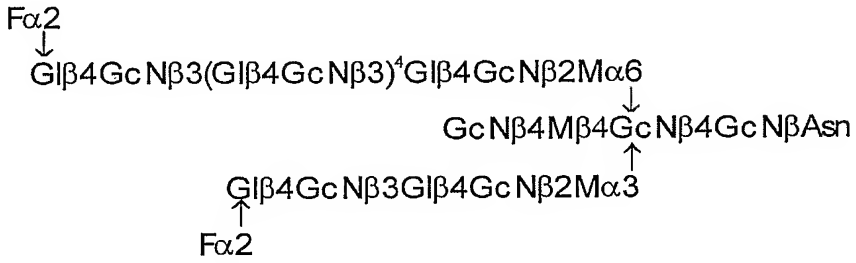
Monofucosyl-monosialoyl foetal erythrocyte antigen (fucosylated branch)



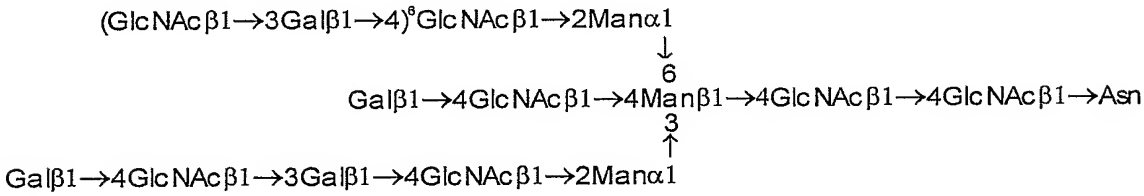
Monofucosyl-disialoyl foetal erythrocyte antigen (disialoyl group on branch)



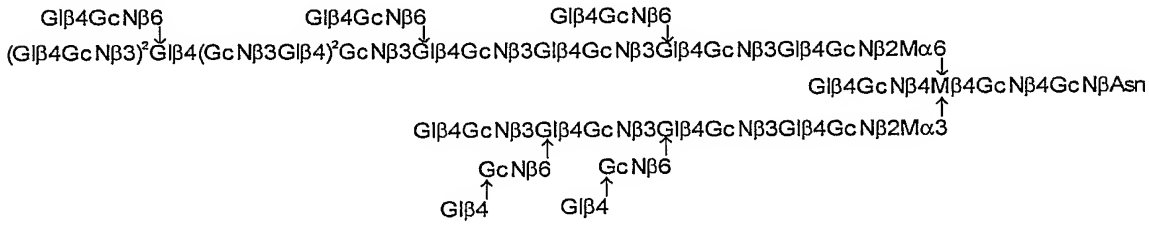
Difucosyl foetal erythrocyte antigen



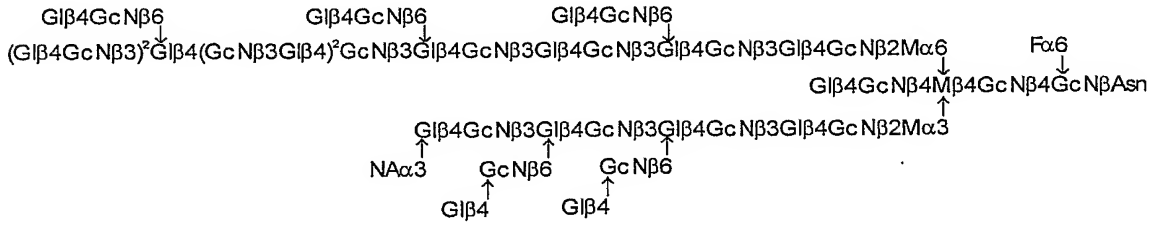
Foetal lactosaminoglycan



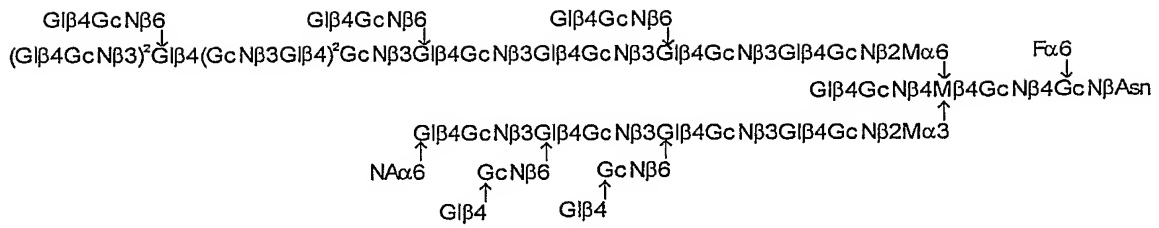
Adult lactosaminoglycan



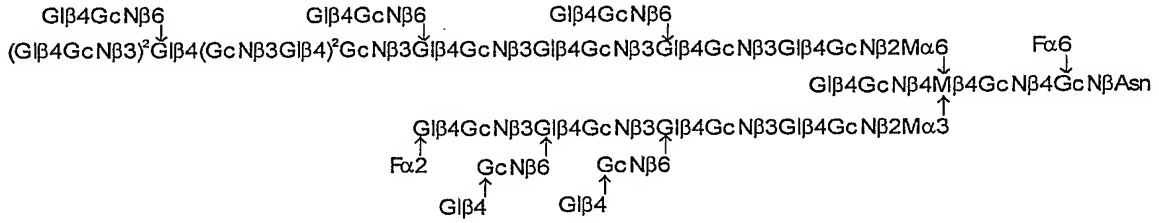
Monofucosyl-monosialoyl adult erythrocyte antigen



Monofucosyl-monosialoyl adult erythrocyte antigen



Difucosyl adult erythrocyte antigen



Key: Gl = Gal, Gc = Glc, GcN = GlcNAc, M = Man, F = Fuc, NA = NeuAc.

In the context of this description of the invention the term "glycolipid" means a lipid containing carbohydrate of amphipathic character including: glycosylated glycerolipids, such as glycosylated phosphoglycerides and glycosylglycerides; glycosylated sphingolipids (neutral glycolipids) such as glycosylceramides or cerebrosides; and gangliosides (acidic glycolipids).

In the context of this description of the invention the phrase "glycolipid-linked antigen" means a lipid containing carbohydrate in which an antigen (typically a protein) is linked to the glycolipid via the carbohydrate portion of the molecule. Examples of glycolipid-linked antigens include GPI-linked proteins.

It will be understood by those skilled in the art that a glycolipid is itself an antigen. The term and phrase are used to distinguish between naturally occurring molecules where the antigen is the glycolipid and naturally occurring molecules where the antigen is linked to the glycolipid via the carbohydrate portion of the glycolipid.

In the context of this description of the invention the term "glycotope" is used to refer to the carbohydrate portion of a glycolipid. The classification of glycolipid antigens is based on the structure of the carbohydrate portion of the glycolipid.

In blood group serology it is known that the terminal sugars of the glycotopes of A-antigens are GalNAcα1-3(Fucα1-2)Galß, and the terminal sugars of the glycotopes of the B-antigens are Galα1-3(Fucα1-2)Galß. Incorporation into the membrane of RBCs of water soluble synthetic molecules of the invention where F is GalNAcα1-3(Fucα1-2)Galß or Galα1-3(Fucα1-2)Galß provides RBCs that are serologically equivalent to A-antigen or B-antigen expressing RBCs, respectively.

The terminal three sugars of the carbohydrate portion of the naturally occurring A- or B-antigen are the determinant of the A and B blood groupings. The terminal four or five sugars of the carbohydrate portion of the naturally occurring A-antigen are the determinant of the A blood sub-groupings A type 1, A type 2, etc.

Accordingly the RBCs incorporating the water soluble synthetic molecules of the invention can be used to characterise and discriminate between blood typing reagents (antibodies) of differing specificity.

It will be understood by those skilled in the art that the carbohydrate portion of a glycolipid may be modified and linked to other antigens by the methods described in the specification accompanying the international application no. PCT/NZ03/00059 (published as WO03087346) and New Zealand provisional application no. 528662 (filed 3 October 2003).

Water soluble synthetic molecules of the invention that exclude a carbohydrate portion are contemplated by the inventors. Antigens other than carbohydrates or oligosaccharides, but with similar physico-chemical properties, may be substituted for F in the "synthetic glycolipids" described.

Water soluble synthetic molecules of the invention that comprise an antigen (F) with differing physico-chemical properties to those of carbohydrates or oligosaccharides are also contemplated by the inventors. Water soluble synthetic molecules comprising these antigens may be prepared by selecting different spacers.

Table 1. Alternative structures of S_1-S_2 for a water soluble synthetic molecule (F-S_1-S_2-L) where F is a glycotope of the A-, B- or H-antigens of the ABO blood groups and L is a phospholipids.

S_1 is selected from:	S_2 is selected from:
$O(CH_2)_3NH$- (3-aminopropyl),	-$CO(CH_2)_3CO$-,
$O(CH_2)_4NH$- (4-aminobutyl), and	-$CO(CH_2)_4CO$- (adipate), and
$O(CH_2)_5NH$- (5-aminobutyl)	-$CO(CH_2)_5CO$-

Table 2. Solubilities of synthetic molecules.			
Structure	Soluble		Used in Examples
	70°C water	70°C PBS	
A_{tri}-sp-Ad-DOPE (I)	n.d..	Yes	Yes
A_{tri}-sp_1sp_2-Ad-DOPE (II)	Yes	n.d.	Yes
A_{tri}-sp-Ad-DSPE (III)	Yes	n.d.	Yes
A_{tri}-sp-lipid (IV)	n.d.	No	No
A_{tri}-PAA-PEA (V)	n.d.	No	No
B_{tri}-sp-Ad-DOPE (VI)	n.d.	Yes	Yes
H_{tri}-sp-Ad-DOPE (VII)	Yes	n.d.	Yes
H_{di}-sp-Ad-DOPE (VIII)	n.d.	Yes	Yes
Galβ-sp-Ad-DOPE (IX)	n.d.	Yes	Yes

The advantages provided by the water soluble synthetic molecules of this invention will accrue when used in the practice of the inventions described in the specifications for the international application nos. PCT/N02/00212 (published as WO03/034074) and PCT/NZ03/00059 (published as WO03087346) and New Zealand provisional application no. 528662 (filed 3 October 2003). The specifications accompanying these applications are incorporated herein by reference.

The invention will now be illustrated by reference to the following non-limiting Examples.

EXAMPLES

Example 1 - Preparation of Water Soluble Synthetic Molecules

Synthesis of activated 1,2-O-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE)

To a solution of *bis*(N-hydroxysuccinimidyl) adipate (A) (70 mg, 205 μmol) in dry N,N-dimethylformamide (1.5 ml) were added DOPE (L) (30 mg, 40.3 μmol) in chloroform (1.5 ml) and triethylamine (7 μl). The mixture was kept for 2 h at room temperature, then neutralized with acetic acid and partially concentrated in *vacuo*.

Column chromatography (Sephadex LH-20, 1:1 chloroform-methanol, 0.2% acetic acid) of the residue yielded the activated lipid (A-L) (37 mg, 95%) as a colorless syrup; TLC (chloroform-methanol-water, 6:3:0.5): Rf = 0.5.

Condensing activated DOPE with aminopropylglycoside

To a solution of activated DOPE (32 mg, 33μmol) in N,N-dimethylformamide (1 ml) were added 30 μmol of aminopropylglycoside (F-S_1) of either Galα1-3(Fucα1-2)Galß trisaccharide (A-glycotope) or GalNAcα1-3(Fucα1-2)Galß trisaccharide (B-glycotope) and 5μl of triethylamine. The mixture was stirred for 2 h at room temperature.

Columns chromatography (Sephadex LH-20, 1:1 chloroform-methanol, and then SiO2, ethyl acetate-isopropanol-water, 4:3:1) of the mixture yielded 85-90% of the synthetic molecules designated A_{tri}-sp-Ad-DOPE (**I**) or B_{tri}-sp-Ad-DOPE (**VI**).

Example 2 - Red Blood Cell Transformation With A- and B-antigen Synthetic Molecules

The water soluble synthetic molecules designated A_{tri}-sp-Ad-DOPE (**I**), A_{tri}-sp_1sp_2-Ad-DOPE (**II**), A_{tri}-sp-Ad-DSPE (**III**), and B_{tri}-sp-Ad-DOPE (**VI**) were prepared according to the method described in Example 1 with necessary modifications.

Washed packed group O red blood cells (RBCs) (3 parts by volume) and the synthetic molecule solution (1 part by volume, varying concentrations) were added to an eppendorf tube. The tube was incubated in a 37°C waterbath for one hour, mixing every 15 minutes. The transformed RBCs were washed 3x with PBS and then suspended in Cellstab at the appropriate concentration for serology testing.

Tube serology and Diamed gel-card results for RBCs transformed with the different synthetic molecules are provided in Table 3. Results for the stability of the RBCs transformed with the different synthetic molecules at different concentrations are provided in Tables 4 to 9.

A and B Antisera:

Antisera	Manufacturer	Batch
Albaclone anti-A	SNBTS	Z0010770 – D.O.E 12.12.04
Bioclone anti-A	Ortho Diagnostics	01102 – D.O.M 16.05.02
Albaclone anti-B	SNBTS	Z0110670 – D.O.E 12.12.04
Bioclone anti-B	Ortho Diagnostics	01103 – D.O.M 16.05.02

Table 3. Comparison of transformation of RBCs using A-antigen synthetic molecules with different non-carbohydrate structures, made to different concentrations.

Synthetic	Conc mg/mL	A Antisera			
		Albaclone anti-A		Bioclone anti-A	
		Tube	Diamed	Tube	Diamed
A_{tri}-sp-Ad-DOPE (I)	0.25	n.d.	4+	n.d.	4+
	0.1	n.d.	4+/3+	n.d.	4+/3+
	0.05	w+	2+	2+	2+
	0.04	w+	n.d.	1+	n.d.
	0.03	0	n.d.	w+	n.d.
	0.02	0	n.d.	0	n.d.
	0.01	0	0	0	0
A_{tri}-sp-Ad-DSPE (III)	0.25	n.d.	0	n.d.	0
	0.1	n.d.	0	n.d.	0
	0.05	0	0	0	0
	0.04	0	n.d.	0	n.d.
	0.03	0	n.d.	0	n.d.
	0.02	0	n.d.	0	n.d.
	0.01	0	0	0	0
A_{tri}-sp_1sp_2-Ad-DOPE (II)	0.25	n.d.	4+	n.d.	4+
	0.1	n.d.	4+	n.d.	4+/3+
	0.05	0	3+	0	3+
	0.04	0	n.d.	0	n.d.
	0.03	0	n.d.	0	n.d.
	0.02	0	n.d.	0	n.d.
	0.01	0	0	0	0
Incubated control	–	0	n.d.	0	n.d.
Bench control	–	0	n.d.	0	n.d.

Abbreviations: n.d. Not determined

Table 4. Stability trial of RBCs transformed with A_{tri}-sp-Ad-DOPE (I) at high concentrations (1 mg/mL, 0.5 mg/mL and 0.25 mg/mL). Agglutination by manual tube serology.

Day	Cell storage solution	Albaclone anti-A			Bioclone anti-A		
		Concentration of Transformation Solution (mg/mL)					
		1	0.5	0.25	1	0.5	0.25
2	Alsevers	4+	4+	4+	4+°	4+°	4+°
	Cellstab	4+	4+	3+	4+°	4+°	4+°
10	Alsevers	3+	2+	2+	4+°	4+°	3+
	Cellstab	4+°	3+°	2+	4+°	4+°	4+°
17	Alsevers	4+	4+	4+	4+°	4+°	4+°
	Cellstab	4+	4+	4+	4+°	4+°	4+°
24	Alsevers	4+	4+	4+	4+	4+	4+
	Cellstab	4+	4+	4+	4+°	4+	4+

Abbreviations: ° splatter

Table 5. Stability trial of RBCs transformed with A_{tri}-sp-Ad-DOPE (I) at low concentrations (0.1 mg/mL, 0.05 mg/mL and 0.025 mg/mL). Agglutination by manual tube serology.

Day	Cell storage solution	Albaclone anti-A			Bioclone anti-A		
		Concentration of Transformation Solution (mg/mL)					
		0.1	0.05	0.025	0.1	0.05	0.025
2	Alsevers	3+/2+	1+	1+/w+	2+	2+/1+	1+
	Cellstab	3+/2+	2+	1+	3+/2+	3+/2+	2+
8	Alsevers	2+	1+	w+	3+/2+	2+	2+
	Cellstab	2+	1+/w+	vw	3+°	2+	1+
15	Alsevers	2+	1+	0	3+	2+	Vw
	Cellstab	4+	w+	0	4+	4+	1+
22	Alsevers	2+	2+	0	3+	2+	w+
	Cellstab	4+	4+	1+	4+	4+	1+
44	Alsevers	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Cellstab	4+	2+	w+	4+	2+	w+

Abbreviations: n.d. Not determined
° splatter

Table 6. Stability trial of RBCs transformed with A_{tri}-sp-Ad-DOPE (I) at high concentrations (1 mg/mL, 0.5 mg/mL and 0.25 mg/mL). Agglutination in Diamed gel-cards.

Day	Cell storage solution	Albaclone anti-A			Bioclone anti-A		
		Concentration of Transformation Solution (mg/mL)					
		1	0.5	0.25	1	0.5	0.25
2	Alsevers	4+	4+	4+	4+	4+	4+
	Cellstab	4+	4+	4+	4+	4+	4+
10	Alsevers	4+	4+	4+	4+	4+	4+
	Cellstab	4+	4+	4+	4+	4+	4+
17	Alsevers	4+	4+	4+	4+	4+	4+
	Cellstab	4+	4+	4+	4+	4+	4+
24	Alsevers	4+	4+	4+	4+	4+	4+
	Cellstab	4+	4+	4+	4+	4+	4+
45	Alsevers	4+	4+	4+	4+	4+	4+
	Cellstab	4+	4+	4+	4+	4+	4+
59	Alsevers	4+	4+		4+	4+	
	Cellstab	4+	4+	4+	4+	4+	4+
73	Alsevers						
	Cellstab	4+	4+	4+	4+	4+	4+
88	Alsevers						
	Cellstab	4+	4+	4+	4+	4+	4+

Where there were insufficient cells for testing, blank spaces have been left.

Table 7. Stability trial of RBCs transformed with A_{tri}-sp-Ad-DOPE (I) at low concentrations (0.1 mg/mL, 0.05 mg/mL and 0.025 mg/mL). Agglutination in Diamed gel-cards.

Day	Cell storage solution	Albaclone anti-A			Bioclone anti-A		
		Concentration of Transformation Solution (mg/mL)					
		0.1	0.05	0.025	0.1	0.05	0.025
2	Alsevers	4+	2+	0	4+	3+	1+
	Cellstab	4+	2+	0	4+	3+	1+
8	Alsevers	4+	3+	0	4+	4+	1+
	Cellstab	4+	3+	0	4+	4+	1+
15	Alsevers	4+	2+	0	4+	3+/2+	1+
	Cellstab	4+	4+	0	4+	4+	1+
22	Alsevers	4+	3+/2+	0	4+	3+	w+
	Cellstab	4+	4+	0	4+	4+	1+
29	Alsevers	4+	2+	0	4+	3+	w+
	Cellstab	4+	3+	0	4+	4+	2+
43	Alsevers	4+	3+	w+	4+	4+	2+
	Cellstab	4+	4+/3+	0	4+	4+	1+
50	Alsevers	4+	3+	w+	4+	4+	2+
	Cellstab	4+	3+	0	4+	4+	1+
57	Alsevers	4+	3+/2+		4+	4+	
	Cellstab	4+	3+	0	4+	3+	w+
63	Alsevers						
	Cellstab	4+/3+	2+	0	4+	3+	0
71	Alsevers						
	Cellstab	4+/3+	2+	0	4+	3+	0
86	Alsevers						
	Cellstab	4+/3+	2+	0	4+	3+	0

Where there were insufficient cells for testing, blank spaces have been left.

Table 8. Stability trial of RBCs transformed with B_{tri}-sp-Ad-DOPE (VI) at high concentrations (1 mg/mL, 0.5 mg/mL and 0.25 mg/mL). Agglutination by manual tube serology.

Day	Cell storage solution	Albaclone anti-B			Bioclone anti-B		
		Concentration of Transformation Solution (mg/mL)					
		1	0.5	0.25	1	0.5	0.25
2	Alsevers	3+	3+	2+	2+	1+	1+
	Cellstab	3+	2+	2+	2+	2+	1+
9	Alsevers	4+	4+	2+	4+	3+	2+
	Cellstab	4+	4+	3+	4+	4+	2+
16	Alsevers	4+	4+	3+	4+	4+	2+
	Cellstab	4+	4+	2+	4+	4+	2+
23	Alsevers	4+	4+	3+	4+	4+	3+
	Cellstab	4+	4+	3+	4+	4+	3+
30	Alsevers	3+	3+	2+	2+	2+	2+
	Cellstab	4+	3+	2+	3+°	3+°	2+
37	Alsevers	3+	2+	1+	3+	2+	1+
	Cellstab	3+	3+	2+/1+	4+°	3+	1+
44	Alsevers	4+	3+	1+	3+	3+	w+
	Cellstab	4+	4+	n.d.	4+	4+	‡
51	Alsevers	3+	3+	2+	4+	3+	2+
	Cellstab	4+	4+	n.d.	4+	4+	2+

Abbreviations: ° splatter

Table 9. Stability trial of RBCs transformed with B_{tri}-sp-Ad-DOPE (**VI**) at high concentrations (1 mg/mL, 0.5 mg/mL and 0.25 mg/mL). Agglutination in Diamed gel-cards.

Day	Cell storage solution	Albaclone anti-B			Bioclone anti-B		
		Concentration of Transformation Solution (mg/mL)					
		1	0.5	0.25	1	0.5	0.25
2	Alsevers	4+	4+	2+	4+	4+	2+
	Cellstab	4+	4+	2+	4+	4+	2+
9	Alsevers	4+	4+	2+	4+	4+	2+
	Cellstab	4+	4+	3+	4+	4+	3+
16	Alsevers	4+	4+	2+	4+	4+	1+
	Cellstab	4+	4+	3+	4+	4+	3+
23	Alsevers	4+	4+	3+	4+	4+	3+
	Cellstab	4+	4+	3+	4+	4+	3+
30	Alsevers	4+	4+	3+	4+	4+	3+
	Cellstab	4+	4+	3+	4+	4+	3+
37	Alsevers	4+	4+	3+	4+	4+	3+
	Cellstab	4+	4+	3+	4+	4+	3+
44	Alsevers	4+	4+	2+	4+	4+	3+
	Cellstab	4+	4+	3+	4+	4+	4+/3+
51	Alsevers	4+	4+	2+	4+	4+	3+
	Cellstab	4+	4+	3+	4+	4+	3+
58	Alsevers	4+		1+	4+		2+
	Cellstab	4+	4+	2+	4+	4+	2+
72	Alsevers	4+		2+	4+		3+
	Cellstab	4+	4+	3+/2+	4+	4+	3+
87	Alsevers						
	Cellstab	4+	4+/3+	1+	4+	4+/3+	2+/1+
116	Alsevers						
	Cellstab	4+	3+	0	4+	4+/3+	1+

Where there were insufficient cells for testing, blank spaces have been left.

Example 3 - Red Blood Cell Transformation with H-antigen Synthetic Molecules

The water soluble synthetic molecules designated H_{tri}-sp-Ad-DOPE (**VII**), H_{di}-sp-Ad-DOPE (**VIII**) and Galβ-sp-Ad-DOPE (**IX**) were prepared according to the method described in Example 1 with necessary modifications.

Washed packed mouse RBCs (3 parts by volume) and the synthetic molecule solution (1 part by volume of varying concentrations) were added to an eppendorf tube. The tube was incubated in a 37°C waterbath for one hour, mixing every 15 minutes. The transformed RBCs were washed 3x with PBS and then suspended in Cellstab at the appropriate concentration for serology testing.

Tube serology and Diamed gel-card results for RBCs transformed with the different synthetic molecules are presented in Table 10. The results show that three sugars (H_{tri}) are required for detection by anti-H IgM.

Antisera	Manufacturer	Batch
Anti-H IgM	Japanese Red Cross	HIRO-75
UEA	Lorne Laboratories	11549E D.O.E. 06.2004
Bio-UEA	EY Labs	201105-2

Table 10. Comparison of transformation of RBCs using H-antigen synthetic molecules with different glycotopes, made to different concentrations.

Synthetic	Conc mg/mL	H Antisera				
		IgM		UEA		Bio-UEA
		Tube	Diamed	Tube T0	Tube T20	Tube
H_{tri}-sp-Ad-DOPE (**VII**)	1	n.d.	n.d.	2+	n.d.	2+
	0.25	4+	3+	n.d.	n.d.	1+
	0.1	3+	2+	n.d.	n.d.	n.d.
	0.05	1+	0	n.d.	n.d.	n.d.
	0.01	0	0	n.d.	n.d.	n.d.
H_{di}-sp-Ad-DOPE (**VIII**)	0.25	0	n.d.	n.d.	n.d.	n.d.
	0.1	0	n.d.	n.d.	n.d.	n.d.
	0.05	0	n.d.	n.d.	n.d.	n.d.
	0.01	0	n.d.	n.d.	n.d.	n.d.
Galβ-sp-Ad-DOPE (**IX**)	0.25	0	n.d.	n.d.	n.d.	n.d.
	0.1	0	n.d.	n.d.	n.d.	n.d.
	0.05	0	n.d.	n.d.	n.d.	n.d.
	0.01	0	n.d.	n.d.	n.d.	n.d.
Human O cells	–	4+	n.d.	1+	2/3+	4+
Incubated control	–	0	n.d.	0	0	n.d.
Bench control	–	0	n.d.	n.d.	n.d.	n.d.

Abbreviations: n.d. Not determined

Example 4 – Insertion of H_{di}-sp-Ad-DOPE (VIII) and Galß-sp-Ad-DOPE (IX) Synthetic Molecules into Murine Red Blood Cells

The water soluble synthetic molecules designated H_{di}-sp-Ad-DOPE (**VIII**) and Galβ-sp-Ad-DOPE (**IX**) were prepared according to the method described in Example 1 with necessary modifications.

Murine RBCs were washed 3x in 1x PBS. 30µl of packed RBCs were combined with 30µl of H_{di}-sp-Ad-DOPE (**VIII**), and 30µl of packed RBCs were combined with 30µl Galß-sp-Ad-DOPE (**IX**), respectively. Both synthetic molecules were at a concentration of 1.0 mg/ml. 30µl of 1x PBS was added to 30µl of packed RBCs to act as the control group. Cells were incubated for 90 minutes in a 37°C shaking water-bath. RBCs were washed 3x in 1x PBS.

Three groups of packed RBCs were incubated with an equal volume of lectin UEA-1 for 30 minutes at room temperature. The lectin was prepared in 1x PBS at a concentration of 0.1 mg/ml. 50µl of a 3% cell suspension was spun for 15 seconds in an Immunofuge at low speed. Results were read by tube serology. The results are presented in Table 11. The results show that neither anti-H IgM nor UEA-1 detects two sugars (H_{di}).

Antisera	Manufacturer	Batch
Biotest anti-H	Biotest AG	
UEA	EY Labs	201105-2

Table 11. Murine RBCs transformed with Galß-sp-Ad-DOPE or H_{dl}-sp-Ad-DOPE, assessed by agglutination.			
Cell Type	Inserted Molecule	UEA-1	Mouse IgM^R
Murine RBC	Galβ (1mg/ml)	0	n.d.
Murine RBC	H_{dl} (1mg/ml)	0	0
Murine RBC	Control (PBS)	0	0
Human RBC	Control(PBS)	4+	3+

Abbreviations: n.d. Not determined

Example 5 - Attachment of Modified Embryos to Transformed Endometrial Cells

Endometrial Cell Transformation

Insertion of water soluble synthetic molecule:

A single cell suspension of endometrial epithelial cells was prepared. The endometrial cells were washed 3x by resuspending in CMF HBSS and centrifuging at 2000 rpm for 3 minutes. The washed cell preparation was resuspended in 50µl of M2.

Micro-centrifuge tubes each containing a 50µl solution of 5M/ml endometrial cells were prepared. To separate tubes of endometrial cells 50 µl of synthetic molecules A_{tri}-sp-Ad-DOPE (**I**) or B_{tri}-sp-Ad-DOPE A (**VI**), or 50 µl M2 were added to the control cells. The cells were incubated for 90 minutes at 37°C on a mixer. The endometrial cells were washed 3x by resuspending in CMF HBSS media and centrifuging at 2000 rpm for 3 minutes. The washed cell preparation was resuspended in 50µl of M2.

Test For Insertion Using Fluorescent Probe:

50 µl of corresponding primary murine monoclonal antibody was added to each tube. Each tube was incubated at room temperature for 10 minutes. Cells were washed 3x in M2 media. 10 µl of mouse anti-IgG FITC was added to each tube. Tubes were incubated at room temperature in dark conditions for 10 minutes. Endometrial cells were mounted on glass slides and viewed under a fluorescence microscope.

Test for Direct Agglutination:

5 µl of each group of cells was placed onto separate microscope slides. To each 5µl drop of cells 5 µl of a corresponding antibody was added. The cells were gently mixed on the slide for 2 minutes. Agglutination was visualised under the microscope. The results are presented in Table 12.

Antisera	Manufacturer	
Bioclone anti-A	Ortho Diagnostics	01102 D.O.M. 16.05.02
Bioclone anti-B	Ortho Diagnostics	Developmental reagent

Table 12. Endometrial cells transformed with A_{tri}-sp-Ad-DOPE (I) or B_{tri}-sp-Ad-DOPE A (VI), as visualised using fluorescence.

Cell Type	Inserted Antigen	1° antibody	Fluorescence score after incubation with IgFITC Probe	Agglutination reaction by microscopic visualisation
Endometrial cells	A_{tri} (1mg/ml)	Anti-A Bioclone	4+	4+
Endometrial cells	B_{tri} (1mg/ml)	Anti-B Bioclone	1+	3+
Endometrial cells	Control (M2 media)	Anti-A Bioclone	0	0

Embryo Modification

Insertion of water soluble synthetic molecule:

The embryo zona pellucida was removed by treating embryos with 0.5% pronase in a 37°C oven for 6 minutes or until all zonas were removed. Micro-drops were prepared by adding 5µl of synthetic molecule A_{tri}-sp-Ad-DOPE (**I**) or B_{tri}-sp-Ad-DOPE (**VI**), at a concentration of 1 mg/mL to a 45µl drop of M2 media overlaid with mineral oil. All embryo groups were incubated in the 50µl micro-drops for 1 hour at 37°C. Embryos from experimental and control groups were washed 3x with M2 media.

Test for Insertion:

Embryos from experimental and control groups were placed into a micro-drop of corresponding antibody and incubate for 30 min at 37°C. Embryos from experimental and control groups were washed 3x with M2 media.

Embryos from all experimental and control groups were placed into micro-drops of anti-mouse Ig FITC (1:50 dilution anti-mouse Ig FITC in M2) and incubated for 30 min at 37°C. Embryos from experimental and control groups were washed 3x with M2 media. Embryos were mounted on microscope slides in a 5µl drop of M2 and the drops overlaid with oil.

The slides were viewed under a fluorescence microscope. Results are presented in Tables 13 and 14. The negative result for transformation with B_{tri}-sp-Ad-DOPE (**VI**) is attributed to a lack of 1° antibody sensitivity.

Table 13. Embryos transformed with A_{tri}-sp-Ad-DOPE (I) as visualised using fluorescence.				
Cell Type	Inserted Antigen	1° antibody	Fluorescence score after incubation with IgFITC Probe	Embryo Morphology 24hr post insertion
Embryos	A_{tri}	Anti-A Bioclone	2+/1+	Appeared viable
Embryos	Control	Anti-A Bioclone	0	Appeared viable

Table 14. Embryos transformed with A_{tri}-sp-Ad-DOPE (I) or B_{tri}-sp-Ad-DOPE (VI), as visualised using fluorescence.				
Cell Type	Inserted Antigen	1° antibody	Fluorescence score after incubation with IgFITC Probe	Embryo Morphology 24hr post insertion
Embryos	A_{tri}	Anti-A Bioclone	2+	n.d.
Embryos	B_{tri}	Anti-B Bioclone	0	n.d.
Embryos	Control (M2 media)	Anti-A Bioclone	0	n.d.

Abbreviations: n.d. Not determined

Enhanced Attachment Transformed Endometrial Cells to Modified Embryos

Modified embryos (BioG-Avidin-BioIgG^B and BioG-Avidin-BioIgM^A) were prepared in accordance with the methods described in the specification accompanying the international application no. PCT/NZ03/00059 (published as WO03087346)

Two concave glass slides were prepared, one with two wells of synthetic molecule A_{tri}-sp-Ad-DOPE (**I**) inserted endometrial cells and the other with two wells of synthetic molecule B_{tri}-sp-Ad-DOPE (**VI**) inserted endometrial cells.

The two groups of embryos were transferred to each of the concave glass slides:

Slide 1 A_{tri}/IgG^B embryos
A_{tri}/IgM^A embryos

Slide 2 B_{tri}/IgG^B embryos
B_{tri}/IgM^A embryos

The embryos were surrounded with endometrial cells. The wells were covered with mineral oil and incubated for 15 minutes at 37°C. Using a wide bore handling pipette each group of embryos were carefully transferred to a fresh drop of M2 media. The embryos were gently washed. The embryos were gently transferred into 2µL of M2 media on a marked microscope slide. Each drop was overlaid with mineral oil

Under a central plane of focus on an Olympus microscope the number of endometrial cells

adhered to the embryos in each group was assessed. The number of cells adhered to each embryo was recorded. Results are presented in Table 15.

Table 15. Endometrial cells transformed with A_{tri}-sp-Ad-DOPE (**I**) or B_{tri}-sp-Ad-DOPE (**VI**), and embryos modified with BioG-Avidin-BiolgG[B] or BioG-Avidin-BiolgM[A]. Assessment by attachment of endometrial cells to embryos.

Cell Type	Transformed endometrial cells	Modified embryos	Average number of endometrial cells attached to modified embryos
Endometrial cells	A_{tri}-sp-Ad-DOPE (**I**)	BioG-Avidin-BiolgG[B]	2.3
		BioG-Avidin-BiolgM[A]	7.25
Endometrial cells	B_{tri}-sp-Ad-DOPE (**VI**)	BioG-Avidin-BiolgG[B]	6.7
		BioG-Avidin-BiolgM[A]	3.4

Where in the foregoing description reference has been made to integers or components having known equivalents then such equivalents are herein incorporated as if individually set forth.

Although the invention has been described by way of example and with reference to possible embodiments thereof it is to be appreciated that improvements and/or modification may be made thereto without departing from the scope or spirit of the invention.

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